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TEMPERATURE DEPENDENCE OF URIDINE TRANSPORT IN QUIESCENT AND SERUM-STIMULATED 3T3 CELLS

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SUMMARY

(1) The kinetics of uptake of uridine into 3T3 cells have been measured as a function of concentration in the temperature range 5–37 °C, for both quiescent and serum-stimulated cells.

(2) The maximum velocity of uridine uptake is increased some ten-fold by adding serum, but the half-saturation concentration is not systematically affected in this temperature range.

(3) A detailed study of the temperature dependence of the maximum velocity of transport in the range 4–43 °C shows that the activation energy of uridine transport is not increased following serum activation.

(4) The data suggest that any change in membrane fluidity that might occur as a result of serum activation does not in itself lead to a more rapid rate of turnover of the individual uridine carriers. It would appear, rather, that there is an increase in the number of functional uridine carriers.

INTRODUCTION

Cells of the 3T3 line of mouse fibroblasts, when grown in cell culture, reach a confluent state after some 3–5 days, at which point cell division ceases and (amongst other things) the activity of certain transport systems shuts off. On adding serum to such density-inhibited cells, uridine and phosphate transport are increased several-fold within minutes, cyclic AMP decreases and subsequently DNA synthesis and cell division takes place [1]. The transport activation reflects an increase in the maximum velocity of the transport process (V) rather than a decrease in the half-saturation parameter (K) [2]. Now, V might increase either as a result of an increase in the number of functionally active transporter molecules, or as a result of the same number of components being able to cross the membrane more rapidly. The latter explanation is indeed plausible since the activation of transport into 3T3 cells by serum occurs in

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the presence of inhibitors of protein synthesis [3], suggesting strongly that no new transporters are synthesized. Furthermore it has been reported that the fluidity of the cell membrane increases after viral transformation [4, 5] and after binding of mitogenic lectins to lymphocytes [6], although these data have recently been questioned [7]. A change in membrane fluidity might similarly accompany stimulation by serum and could then lead to an increased rate of transmembrane flow of carrier molecules or to more rapid rates of conformation changes of larger fixed transporters. That changes in the state of fluidity modify membrane transport and membrane-associated enzymes and receptors has been well documented in a number of systems [8–12]. If the mechanism whereby serum rapidly increases transport rates is an increase in membrane fluidity, serum should, however, necessarily reduce the activation energy of the transport process. Our experiments (carried out on the uridine transport system of 3T3 cells) were designed to test whether this predicted change of activation energy in fact occurs. They do not bear out this prediction.

METHODS

Balb/c/3T3 cells were maintained in reinforced Eagles medium supplemented with 10 % serum, 100 units/ml of penicillin and 100 μg/ml of streptomycin. Transport was measured as described below on cells still attached to 30-mm Nunc petri dishes. A dish contained, on average, 110 μg of cellular protein. Cultures of confluent cells were used 3–5 days after the last previous change of medium. Activation was performed by incubating the cells with 2 ml of fresh 10 % foetal calf serum medium for 30 min at 37 °C, taking care to maintain the pH of the medium in the range 6.9–7.2 during this time. Control (quiescent) cells were treated exactly as activated cells except that Eagles medium with no added serum was added to the final incubation medium. After activation or sham activation the incubation medium was removed, fresh medium pre-equilibrated at the desired temperature was added and left an additional 2 min. Then a solution of [^{3}H]uridine was added (the same total number of radioactive counts being added to each dish) and uptake into the acid-soluble pools was determined as previously described [13]. Direct chromatographic analysis has shown that more than 85 % of the added uridine label is, at 37 °C, found in the acid-soluble pool, a negligible amount of this being free uridine. Uptake of labelled uridine was linear over the time periods studied (5 min at 37 °C, increasing to 60 min at 5 °C). The concentration of uridine covered the range 0.05–500 μM. For each set of data a zero time blank was taken by exposing cultures for as short a time as possible to labelled uridine. Such blanks were seldom more than one-third of the test values, and generally one-tenth to one-twentieth of such values.

The data on the concentration dependence of uptake were analyzed according to a curve-fitting procedure (programme NONLSQ of the Hebrew University computing centre) using the equation

$$\text{cpm/dish per min} = V/(K+S)+B \qquad (1)$$

where B is the slope of the linear portion of the curve of uptake against high substrate concentration, K is the half-saturation concentration and V is the maximal value of the saturable component of the quantity cpm/dish per min multiplied by the substrate concentration, S.

Since the experiments were performed at a constant total radioactivity of uridine per dish (2.5 μCi/ml), the measured uptake as cpm multiplied by the substrate concentration gives the amount of uridine entering per dish. Dividing both sides of the conventional equation describing uptake for a saturable component in parallel with a non-saturable component, namely

$$v = \frac{VS}{K+S} + BS \tag{2}$$

by S, gives Eqn 1, a preferred form since it is in terms of directly measured variables and avoids complications of weighting.

RESULTS

Initially, we measured the kinetic parameters of uridine transport in quiescent and serum-stimulated cells at different temperatures. In all cases, the uptake of uridine

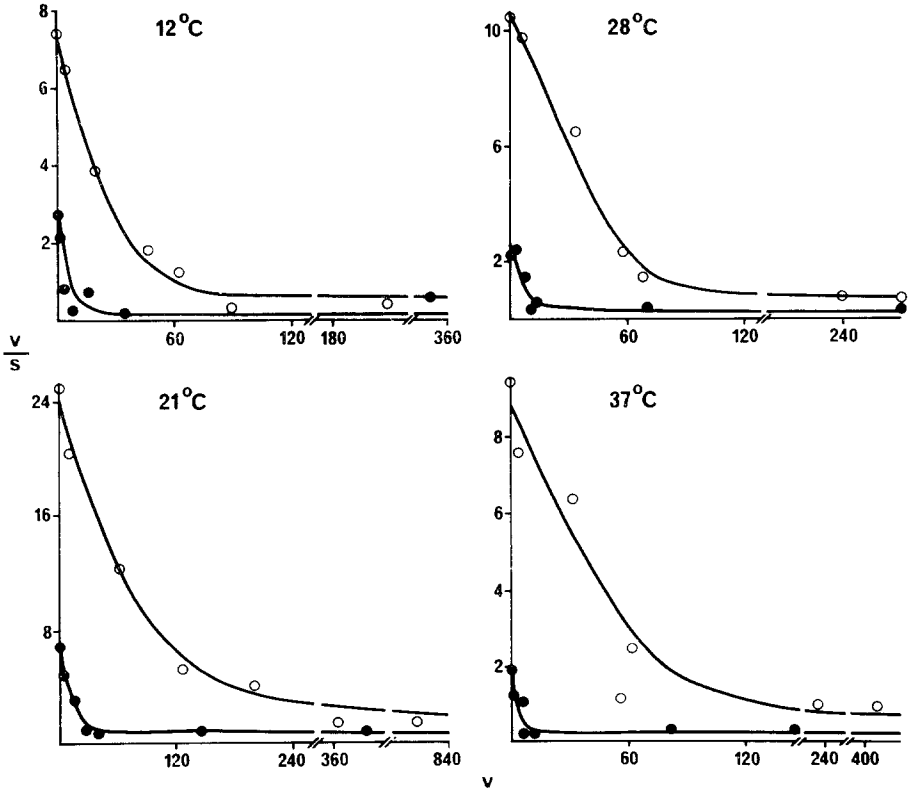

Fig. 1. Hofstee plot (v/S against v) of uridine uptake by quiescent and serum-stimulated Balb/c/3T3 cells as a function of substrate concentration at four different temperatures. Uptakes performed as described in the text, a constant total amount of uridine label (5 μ Ci) being added to each dish. The ordinate gives the cpm per dish (in thousands of counts) during the uptakes periods of 5, 10, 15 and 30 min at the temperatures of 37, 28, 21 and 12 °C, respectively. The abscissa gives this uptake at each point multiplied by the respective uridine concentration in molars. The kinetic parameters V, K and B fitted from these data are recorded in Table 1.

TABLE I

KINETIC PARAMETERS OF URIDINE UPTAKE BY Balb/c/3T3 CELLS AS A FUNCTION OF SERUM ACTIVATION AND TEMPERATURE

K is the half-saturation concentration and V the maximum velocity of the saturable component of uridine uptake, B is the slope of the linear component of the curve of uptake against concentration.

Serum	Temperature (°C)	$K \pm$S.E. (μM)	$V \pm$S.E. (pmol · dish^{-1} · min^{-1})	$10^9 \times B \pm$S.E. (dish^{-1} · min^{-1})
+	37	8.8±3.4	12.2±4.4	15 ±17
0	37	4.4±2.9	1.15±0.72	6.1± 4.7
+	28	6.7±0.7	4.95±0.48	5.6± 2.0
0	28	4.3±1.8	0.68±0.26	3.4± 1.7
+	21	4.2±0.9	3.4 ±0.66	12 ±4.3
0	21	3.0±1.0	0.62±0.19	4.3±1.5
+	12	5.3±0.9	1.0 ±0.15	2.4±0.8
0	12	1.3±0.8	0.09±0.05	2.0±0.8
+	5	8.9±3.4	0.3 ±0.05	*
0	5	16.8±7.6	0.07±0.02	*

* These data were obtained on a different batch of cells from those above, several months later. Insufficient data were available to allow the calculation of the parameter B, but the experiment indicates that no significant difference between the parameter K for quiescent and serum-stimulated cells develops as the temperature is lowered.

followed clearly a complex kinetic (Fig. 1) suggesting the presence of a saturable as well as a linear component of entry. Statistical analysis of the data (see Methods section) yielded the results recorded in Table I. From this Table we can conclude that: (a) the non-saturable component was increased somewhat by serum, but never significantly so; (b) K is not decreased by serum; at 12 °C it indeed increased slightly, an effect which is opposite to that to be expected if a more effective transport system is revealed by serum; (c) V is very significantly increased on serum activation in all cases.

Neither in the presence nor in the absence of serum is K systematically affected by temperature. V, in contrast, clearly increases with increasing temperature but the steepness of this increase is, if anything, greater in the presence of serum (that is, the activation energy of transport is increased following activation by serum rather than decreased, as might be expected to result from a change in fluidity).

The latter finding argues against a change in fluidity as the basis for the increase in transport into cells stimulated to proliferate. In order to corroborate this conclusion a more detailed study of the effects of temperature on V was carried out. The simple approach of getting V directly by measuring transport at very high substrate concentrations is here unreliable since the substantial linear component of uridine uptake could overshadow any saturable component at high concentrations. To overcome this difficulty we have derived V/K_m by combining data obtained at both high and low substrate concentration, as follows.

Data were obtained at uridine concentrations of 0.05 and 500 μM, being some 1/100 and 100 times the average value of K_m (from Table I), at different temperatures in the range 4–43 °C. Considering Eqn 1, at low concentrations of uridine compared

to K_m, the counts taken up per min are given by $(V/K_m) \cdot B$. At high concentrations of uridine (relative to K_m) the first term of Eqn 1 tends to zero, so the rate of entry of counts is given by B. If the entry of uridine is studied, therefore, both at very high and at very low concentrations of uridine, and the counts subtracted from one another, V/K_m and B can be separately evaluated. Since K_m does not vary significantly with the state of stimulation of the cells (Table 1) the variation in V/K_m indicates how V is affected by serum stimulation at each temperature.

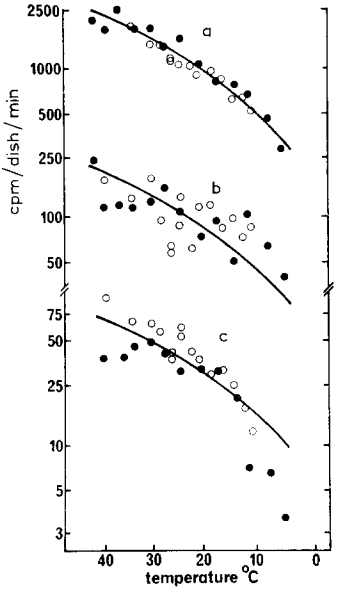

Fig. 2. Dependence on temperature of the components of uridine transport into quiescent and serum-stimulated Balb c/3T3 cells. The values of V/K_m for serum-stimulated cells (a) and for quiescent cells (b) and the value of B for quiescent cells (c), as calculated in the Results section, are presented in a logarithmic scale in units of cpm/dish per min, against the reciprocal of the absolute temperature. (The temperatures are shown in °C for clarity.) Data from two experiments on separate batches of cells are combined as indicated by the different symbols. Each point represents the average of a triplicate transport determination. Experimental details were as described in the Method section.

Considering first the data on serum-stimulated cells (Fig. 2a), it is clear that the dependence of the logarithm of V/K_m on temperature is not linear. It might be that more than one step is involved in the transport process mechanism and that different steps become, in turn, rate limiting as the temperature is altered. Indeed, numerous phase transitions might occur in the membrane phase [11], these changes modifying the rate of transport by the membrane transporter. Whatever the underlying mechanism, however, the curve drawn freehand through the points serves to define the temperature dependence of this transport system. (An explicit calculation of activation energies is clearly not justified, owing to the non-linearity of the plot.)

To see if the dependence on temperature of uridine transport in quiescent cells is or is not significantly different from that in stimulated cells, the curve obtained from Fig. 2a has been transposed downwards onto Fig. 2b, the data for quiescent

cells, so as to go through the average of the points at 26 °C, this being the middle of the temperature range. The scatter of the points is here large (since the non-saturable component of uptake forms a substantial fraction of the total uptake in these quiescent cells and this component must be subtracted from the total to obtain the saturable component), but clearly the curve obtained for the points of Fig. 2a is a reasonable fit for those of Fig. 2b. If anything, the temperature dependence of the transport rate in the quiescent cells is less than that of the stimulated cells, i.e. the activation energy is somewhat smaller, the reverse of what would be expected if a phase change in the membrane were directly responsible for the increased transport rates. In contrast, the curve taken from Fig. 2a when fitted to the points at 26 °C for the non-saturable component in Fig. 2c fails to describe these data adequately at the lower temperatures. The greatest activation energy is found for the non-saturable component.

DISCUSSION

Our results indicate that there is no substantial change in the activation energy of the maximum velocity of uridine transport following serum stimulation of quiescent cells. Comparable results have been reported by Kletzien and Perdue [14] ,who found the same energy of activation for sugar transport when comparing growing and confluent cultures of chick embryo fibroblasts, and such chick cells after transformation by a Rous sarcoma virus strain. Thus one can conclude that although there may be marked changes in the fluidity of the cell membrane, these are not manifested directly as increases in the transport rates. It has previously been shown that the stimulation of transport takes place rapidly in the presence of inhibitors of protein synthesis [3]. Taken together, these facts suggest a model in which the membrane component responsible for uridine transport is present at all times. In quiescent cells, this component is present in a non-functional state either in a cytoplasmic precursor pool or else in the membrane, but in a non-functional form. On adding serum, the uridine transport system is rendered functional once more. It is possible that cyclic AMP is involved in controlling the functional state of the uridine transporter [12]. One might generalize from our results and from previous data showing a dissociation between the early events after serum stimulation [3, 13] and conclude that the initiation of growth involves, rather than a single underlying event, a series of parallel events which are activated by specific and independent mechanisms.

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